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Research Article

Influence of Dietary Supplementation with Prebiotic, Oregano Extract, and Vitamin E on Fatty Acid Profile and Oxidative Status of Rabbit Meat

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The effect of dietary supplementation with vitamin E, oregano, and prebiotic on fatty acids and oxidative profiles of rabbit meat (loin and hind leg) was evaluated. New Zealand white rabbits weaned at 30 days of age were fed with one of six diets until 80 days of age: standard diet including $\omega 3$ polyunsaturated fatty and conjugated linolenic acids sources (S) and five diets adding vitamin E (150 ppm, E), oregano water extract (2 g/kg feed diet, O), prebiotic (THEPAX® 1.5 g/kg feed diet, T), vitamin E plus prebiotic (TE), and oregano water extract plus prebiotic (TO), respectively. The lipid oxidative status (TBARS) showed lower values with respect to S, mainly when vitamin E was administered. In particular, all the experimental diets decreased TBARS values with respect to the control group in the loin, but no effect was found in the hind leg. In all feed samples, the amounts of fatty acid classes increased in the following order: polyunsaturated fatty acids > monounsaturated fatty acid > saturated fatty acid. The dietary supplementations did not affect the fatty acid composition of meat. The experimented diets compared to the control were not able to provide a selective increase of bioactive fatty acid in meat samples; however, the six nutritional strategies led to highly nutritional rabbit meat with an interesting value of the $\omega 6/\omega 3$ ratio.

1. Introduction

Italy is the main producer of rabbit meat in the European Union, with a production of approximately 230 t/year [1]. However, this production does not cover the domestic demand, which has been increasing since the last decade [2]. This particular meat is widespread in each region all over the country and it represents a traditional dish highly digestible and tasty. In addition rabbit meat offers excellent nutritive proprieties [3–7]. It is a lean meat rich in proteins of high biological values, low cholesterol content, and a noticeable quantities of polyunsaturated fatty acids (PUFA), which are strictly involved in human health [8–12]. It is worth noting that the PUFA content ranged from 35 to 40% of total fatty acids in rabbit meat, whereas it accounted for 4.0% of total fatty acid in the muscle of cattle, 5.2% in lamb meat, and 17.7% in pork meat [13]. Taking into account the fact that animal feed is able to exert a considerable influence on the meat composition of monogastric livestock, not only as the amount of fat but even on its fatty acids composition, rabbit meat could represent an excellent base to produce a specific functional food providing an even healthier food for human consumption. In fact, dietary fatty acids are incorporated unchanged into adipose tissue of monogastrics, whereas in ruminants feed they are hydrogenated in the rumen [14]. Despite several studies reporting the influence of feeding with plant extract, vitamin E, and prebiotic compounds on quality parameters of rabbit meat [15–25], little information

Table 1: Chemical composition (g/kg) of the experimental diets.

	Diet					
	S	E	O	T	TE	TO
Dry matter	884.2	862.3	900.2	910.0	888.2	895.0
Crude protein	165.2	156.5	155.9	157.6	154.0	159.1
Ether extract	40.3	41.9	41.5	40.6	42.0	41.8
Starch	120.5	110.8	119.8	127.5	120.1	126.2
NDF	410.5	395.2	430.9	425.0	420.2	419.2
ADF	214.3	218.0	241.2	224.5	229.3	224.8
Lignin	47.5	44.1	55.0	56.0	49.8	48.7
Ash	85.3	83.9	82.0	84.6	83.7	84.9
Ca	12.3	14.2	9.0	9.9	10.2	11.5
Na	1.6	1.7	1.8	1.6	1.8	1.6
P	5.2	5.5	5.4	5.7	5.3	5.5
DE (Mj/kg DM)	10.5	10.6	10.5	10.4	10.7	10.6

Values are the mean of four different determinations. S = standard diet (0.5% CLA. 3% ω3 source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 2% oregano extract; T = standard diet + 1.5 g/kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/kg prebiotic THEPAX; TO = standard diet + 2% oregano extracts + 1.5 g/kg prebiotic THEPAX; DE = digestible energy, calculated according to Villamide et al. [28].

concerning the effect of supplementation diet with these additives on rabbit meat fatty acids composition is available [26, 27].

Previous studies confirmed the possibility of modifying the fatty acid profile of rabbit meat by dietary means. Bernardini et al. [29] considering the effect of dietary PUFA ω3/PUFA ω6 ratio on fatty acid composition of liver, meat, and perirenal fat in rabbits observed how it possible to enhance the PUFA content of rabbit meat, with benefits to the health of consumers. After the experimental feed enrichment using conjugated linoleic acids (CLAs) supplementation, it has been observed that the concentration of CLA in rabbit meat lipids increased with increasing CLA content in the diet. On the other hand, the high PUFA amount, naturally present or supplied by feed diets, could expose the lipid fraction of rabbit meat to oxidation process compromising the technological stability of the product. Meat oxidation leads to a reduction of shelf life due to the rancidity and color corruption [2]. In this context, dietary supplementation with a proper amount of antioxidants could provide a good alternative to enhance the oxidative stability of meat with high content of PUFA [30].

Alternatively to the employment of vitamin E, recent researches have suggested that supplementation with phenolic compounds may have beneficial effects on the antioxidant defense system of the animal and protects cell biological membranes against lipid oxidation also preserving the welfare and food quality. Herbs and spices extracts contain phytochemical compounds that are a valid source of natural antioxidant as phenolic compounds and tannins. These natural constituents are involved to extend meat shelf life and retard oxidation activities.

Besides vitamins and plant extract, newest research have focused the attention on prebiotic compounds. They are nondigestible substances, which are not absorbed by the body but utilized by the intestinal flora. Prebiotics provide a beneficial physiological effect on the host, by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria [31]. They are also associated with improvement of animal performance and the increase of nutrient availability, which, in turn, positively affect the quality of animal products [32].

In this scenario, the present work was aimed to acquire a deeper knowledge about of effect of dietary vitamin E, oregano water extract, and prebiotic compounds on fatty acid profile and oxidative stability of rabbit meat. In particular, the impact of different feeding will be evaluated in order to establish if the addition of oregano extract, prebiotic compounds, and vitamin E into the diet enriched of bioactive fatty acids, such as CLA and PUFA ω3, could promote the enhancement of such fatty acids content on rabbit meat. This way could be useful in developing a value-added meat product with beneficial health properties for humans.

2. Materials and Methods

2.1. Sampling and Experimental Diets. This study was part of a project supported by Italian Ministry of Economic Development and was carried out in collaboration with important partners in the Italian rabbit production chain. The research was carried out at the experimental farm of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia (Italy). A total number of 240 New Zealand white rabbits were weaned at 30 days and randomly allocated to six dietary groups, homogeneous for sex and weight (40 rabbits/group). Rabbits were individually housed in wire cage (60 × 25 × 33 cm). Stainless steel nipples were used for drinking and feeders were supplied for each cage (ad libitum). The rearing temperature and light management were, respectively, 15–18°C and 16 h light/8 h darkness.

The feeding period continued up to 80 days. All diets, provided by Mignini and Petrini (Petrignano di Assisi, Italy) were isonitrogenous and isoenergetic.

Chemical composition (g/kg) of the experimental diets was reported in Table 1. In particular, the control group (S) was fed a standard diet containing alfalfa meal, sunflower

seed meal, wheat bran, barley, and sugar beet pulp as main ingredients and an integration of vitamin E (50 ppm), CLA (5 g/kg fed diet, soy oil extract), extruded linseed (30 g/kg fed diet, OmegaLin® Mignini and Petrini), and vitaminic complex (5 g/kg fed diet Vitcon®).

The O supplemented group received a standard diet plus oregano water extract Phenbiox® (2 g/kg fed diet) whereas the TO group received a standard diet plus oregano water extract Phenbiox (2 g/kg fed diet) and prebiotic compound THEPAX (1.5 g/kg fed diet, inactivated *Saccharomyces cerevisiae*). The E supplemented group received a standard diet plus vitamin E (150 ppm) whereas the T supplemented group received a standard diet plus prebiotic compound THEPAX (1.5 g/kg fed diet). Finally, the TE supplemented group received a standard diet plus prebiotic compound THEPAX (1.5 g/kg fed diet) and vitamin E (150 ppm).

The oregano extracts were provided by Phenbiox (Calderara di Reno, Bologna, Italy), while the prebiotic was inactivated yeast provided by THEPAX (THEPAX, Doxal Italia, Sulbiate, Italia).

Eight rabbits for each group were sacrificed at 80 days of age and the carcasses were brought at the Department of Agricultural, Food and Environmental Sciences of the University of Perugia. After chilling (24 h at +4°C) from carcasses were isolated the loin *(longissimus dorsi)* and the total hind legs muscles *(semitendinosus, semimembranosus, gracilis and adductor, gluteobiceps, biceps femoris, quadriceps femoris, vastus lateralis, vastus intermedius, rectus femoris, and tensor fasciae latae)*.

These two major cuts with high commercial value were chosen and stored at −20°C for 2 wks.

2.2. Chemical Analysis of Experimental Diets. Dry matter was determined by oven drying at 105°C overnight [33]. Crude protein was measured by a Kjeldahl nitrogen analysis [33]. Ash content was determined by combusting for 3 h at 550°C. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) content were determined by van Soest et al. [34]. Lipids were extracted by diethyl ether using a Soxhlet apparatus.

2.3. Chemical Analysis and Oxidative Status of Meat. Proximal composition (moisture, protein, lipid, and ash) was performed, according to AOAC [35] from lyophilized loin and hind leg meat. The moisture content was determined by oven drying meat samples (125°C for 24 h) (method 950.46). The fat content was determined gravimetrically using ether solvent extraction (method 960.30). The protein content was obtained multiplying the total Kjeldahl nitrogen (method 992.15) with a coefficient factor of 6.25. The ash content was determined using a muffle furnace at 600°C (method 923.03) [35].

The extent of muscle lipid oxidation was evaluated on thawed samples, with a spectrophotometer set at 532 nm (Shimadzu Corporation UV- 2550, Kyoto, Japan) that measured the absorbance of thiobarbituric acid-reactive substances (TBARS) and a 1,1,3,3-tetraethoxypropane calibration curve [36]. Oxidation products were quantified as malondialdehyde equivalents (mg MDA/kg muscle).

2.4. Extraction of Total Lipids and Analysis of Fatty Acid Profile of Diets and Meat. Eight fresh loin and hind legs as concerns each feed group were randomly collected (four from male and four from female rabbits) and utilized for lipid extraction. An aliquot (20 g) of each muscle tissue and two of each diets (20 g) was homogenized in chloroform : methanol (1 : 2, v/v) and the lipids were extracted according to Bligh and Dyer [37].

Fatty acid methyl esters (FAMEs) were obtained from total lipids through alkaline transmethylation [38]. The qualitative analysis of FAMEs was carried out using a Focus gas chromatograph (Thermo Electron Corporation, West Palm Beach, FL, USA) equipped with a CP-Sil88 fused silica capillary column (100 m × 0.25 mm i.d., film thickness 0.2 μm, Chrompack, Middelburg, The Netherlands) and a quadrupole mass detector (FocusDSQ). The carrier gas was helium at a flow rate of 1.6 mL/min; the oven temperature program started from 160°C, raised to 240°C at a rate of 4°C/min, and remained at 240°C for 10 minutes. The injector temperature was 260°C. The sample was injected into a split/splitless system. The ion source temperature of the mass detector was set at 260°C. The mass spectrum was acquired using Xcalibur Data System ver. 1.4. Peaks were identified by comparison with known standards and using the NIST mass spectral database. The quantitative analysis of FAMEs was performed by means of gas chromatography using a CP-9002 apparatus (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector (FID) and the same column and operative conditions reported above. The temperature of the detector was set at 260°C. A Supelco (Bellefonte, PA, USA) standard solution containing a mixture of 37 FAMEs was used for identification of peaks and for the calculation of correction factor of the individual fatty acid peak areas. Fatty acid compositions (wt%) were calculated by the corrected peak area normalization method. The concentrations of fatty acids in mg/100 g of wet feed and mg/100 g of meat were measured against nonadecanoic acid methyl ester (C19:0) as an internal standard.

2.5. Statistical Analysis. The data collected were grouped into feed samples, in order to point out the effect of different feeds on fatty acids profile of loin and hind legs. All data were presented as group mean values ± standard deviation (SD, $n = 8$).

The statistical analysis of data was performed by one way ANOVA carried out with software R Project for Statistical Computing (R Foundation for Statistical Computing, Wien, Austria). To explain significant means differences, post hoc analyses have been performed through Tukey test, suitably fitted to the repeated measure design (TukeyC R package). Differences were detected in order to get an overview of the differences in the fatty acid composition among the fresh meat. For all statistical analyses, significance was declared at $p < 0.01$.

3. Results and Discussion

The present study is a part of a wide research aimed to evaluate the effect of the dietary supplementation of oregano

Table 2: Lipid content and fatty acid composition of pellet feeds belonging to different dietary groups.

	Diet					
	S	E	O	T	TE	TO
Lipid content (g/100 g of wet feed)	4.5 ± 0.2	4.7 ± 0.3	4.6 ± 0.2	4.2 ± 0.1	4.3 ± 0.2	4.3 ± 0.3
Fatty acid (% of total fatty acids)						
C16:0	13.9 ± 0.6	13.1 ± 0.4	12.1 ± 0.8	13.3 ± 0.5	13.6 ± 0.4	14.4 ± 0.2
C18:0	6.7 ± 0.3	6.7 ± 0.5	6.5 ± 0.3	6.5 ± 0.2	6.7 ± 0.3	5.9 ± 0.8
C20:0	0.9 ± 0.3	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.6 ± 0.1
C21:0	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.2
C24:0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	tr	tr	tr
$\sum SFA$	21.7 ± 0.6	20.5 ± 0.4	19.3 ± 0.6	20.4 ± 0.3	20.7 ± 0.3	20.9 ± 0.6
C18:1 Δ^{9c} + C18:1 Δ^{11c}	25.2 ± 0.5	24.1 ± 0.4	24.5 ± 0.5	23.9 ± 0.6	23.3 ± 0.8	25.2 ± 0.4
C20:1 Δ^{11c}	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
$\sum MUFA$	26.0 ± 0.9	24.5 ± 0.5	25.1 ± 0.5	24.2 ± 0.5	23.8 ± 0.3	25.6 ± 0.6
C18:2 $\Delta^{9c,12c}$ [ω6]	25.4 ± 0.5	26.7 ± 0.5	26.0 ± 0.5	27.8 ± 0.8	27.6 ± 0.6	28.9 ± 0.9
C18:3 $\Delta^{9c,12c,15c}$ [ω3]	11.8 ± 0.2	12.7 ± 0.7	13.1 ± 1.3	13.6 ± 1.5	14.1 ± 1.5	12.2 ± 0.2
C18:2 $\Delta^{9c,11t}$ [CLA]	4.9 ± 0.3	4.4 ± 0.2	5.1 ± 0.6	4.4 ± 0.3	4.6 ± 0.2	4.6 ± 0.4
C18:2 $\Delta^{10t,12c}$ [CLA]	4.5 ± 0.4	4.2 ± 0.3	5.0 ± 0.5	4.2 ± 0.2	4.3 ± 0.4	4.4 ± 0.3
C18:2 $\Delta^{9t,11c}$ [CLA]	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
C18:2 $\Delta^{10c,12t}$ [CLA]	1.9 ± 0.4	1.4 ± 0.2	2.0 ± 0.4	1.6 ± 0.3	1.7 ± 0.2	1.6 ± 0.4
$\sum CLA$	11.9 ± 0.9	10.5 ± 0.7	12.8 ± 1.1	10.5 ± 0.4	11.1 ± 0.8	11.2 ± 0.4
$\sum PUFA$	49.6 ± 0.8	51.3 ± 0.9	52.7 ± 1.4	52.0 ± 0.8	52.8 ± 0.9	52.3 ± 0.6
PUFA ω6/PUFA ω3	2.1 ± 0.2	2.2 ± 0.3	2.0 ± 0.1	2.0 ± 0.2	2.0 ± 0.1	2.4 ± 0.3

Values are given as means ± standard deviation ($n = 4$); trace (tr), lower than 0.1%; S = standard diet (0.5% CLA. 3% ω3 source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 2% oregano extract; T = Standard diet + 1.5 g/kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/kg prebiotic THEPAX; TO = standard diet + 2% oregano extracts + 1.5 g/kg prebiotic THEPAX.

aqueous extract, vitamin E, and inactivated S. *cerevisiae* yeast on live performance, health status, and meat quality of growing rabbits.

The lipid average expressed on a wet weight basis and the fatty acid composition (weight % of total fatty acids) of feeds belonging to different dietary groups were reported in Table 2.

The lipid content did not statistically change among the feeds and it ranged from 4.2 to 4.7 g/100 g of feed. Similarly, the qualitative fatty acid composition of the diet did not change according to the feeding trials. In all feed samples, the amounts of fatty acid classes increased in the order PUFA > monounsaturated fatty acid (MUFA) > saturated fatty acid (SFA). The levels of linoleic (C18:2 ω6) and oleic (C18:1 Δ^{9c}) acids were comparable and they prove to be the major fatty acids in all feeds.

The SFA fraction was mainly formed by palmitic (C16:0) and stearic (C18:0) acids whereas the MUFA fraction mainly contained oleic (C18:1 Δ^{9c}) and elaidinic (C18:1 Δ^{11c}) acids.

As regards the PUFA fraction, it is noteworthy to underline that all tested feeding trials presented higher content of PUFA (49–52% of total fatty acids, 600 mg/100 g pellet) compared to values reported in the literature (ranging from 30 to 40% of total fatty acids) [39]. Besides C18:2 ω6, the PUFA fraction was mainly formed by α-linolenic acid (C18:3 ω3) and CLA isomers, especially *cis*-9, *trans*-11 CLA and *trans*-10, and *cis*-12 CLA.

The chemical composition and TBARS of loin and hind leg rabbit meat are reported in Table 3.

In this study the proximate composition of meat did not change according to different dietary treatments; in a previous study we observed a reduction of the protein content of *longissimus dorsi* muscle in rabbits treated with oregano (2%) with respect to control group [25]. The lipid amount was slightly higher in TE, but not statistically different with respect to the other experimental groups. The TBARS values were lower in all experimental groups than control in loin muscle; the lowest values were observed in E and TE groups. This last results is probably due to a cooperation between vitamin E and THEPAX. In fact the yeast administration improves the rabbit microbiota and the activities of digestive enzymes, promoting the digestibility of the feed and nutrient availability [40, 41]. In this context the antioxidants intake (i.e., vitamin E) could be promoted.

In agreement, Wang et al. [42], reported a dose-depended reduction in serum and liver MDA content of juvenile starry flounder supplemented with yeast prebiotic (Grobiotic®-A) at different concentration (from 0 to 2.0%).

The lipid oxidative status of hind leg showed an absence of significant differences, probably due to the high variability of lipid content (from 2.41 to 3.18 g/100 g). In fact it is possible to note that the S and TO group had similar TBARS values (0.38 and 0.37 mg MDA/kg of meat) despite the lipid % being different (2.41 versus 3.18%). Considering that the TO diet provided a higher amount of antioxidant compounds [32] which balance the lipid oxidation, we can assume a better lipid oxidative status in TO group when the lipid content is equal.

The fatty acid compositions (weight % of total fatty acids) of rabbit loin and hind leg were reported in Tables 4 and 5, respectively. The dietary supplementation with prebiotic

Table 3: Effect of the dietary treatment on the proximate composition (g/100 g meat) and TBARS (mg MDA/kg meat) contents of rabbit loin and hind leg.

	Diet					
	S	E	O	T	TE	TO
Loin						
Moisture	74.1 ± 0.9	74.4 ± 0.6	74.2 ± 0.7	74.1 ± 1.1	74.9 ± 1.0	74.1 ± 0.9
Protein	24.0 ± 0.6	24.1 ± 0.8	23.9 ± 0.5	23.4 ± 0.3	23.2 ± 0.9	23.8 ± 0.5
Lipid	0.46 ± 0.2	0.48 ± 0.3	0.44 ± 0.5	0.45 ± 0.8	0.51 ± 0.7	0.47 ± 0.5
Ash	1.35 ± 0.11	1.30 ± 0.13	1.28 ± 0.09	1.37 ± 0.16	1.33 ± 0.14	1.44 ± 0.05
TBARS	0.26 ± 0.08^{c}	0.15 ± 0.07^{a}	0.20 ± 0.03^{b}	0.22 ± 0.09^{b}	0.17 ± 0.07^{a}	0.21 ± 0.04^{b}
Hind leg						
Moisture	74.3 ± 0.4	74.5 ± 0.5	74.7 ± 0.6	74.5 ± 0.7	74.7 ± 0.5	73.7 ± 0.4
Protein	22.0 ± 0.4	21.2 ± 0.3	21.1 ± 0.7	21.2 ± 0.5	21.1 ± 0.4	21.7 ± 0.7
Lipid	2.41 ± 0.21	2.74 ± 0.30	2.77 ± 0.19	2.74 ± 0.24	2.77 ± 0.15	3.18 ± 0.24
Ash	1.25 ± 0.09	1.57 ± 0.11	1.47 ± 0.10	1.57 ± 0.11	1.47 ± 0.17	1.44 ± 0.08
TBARS	0.38 ± 0.05	0.32 ± 0.03	0.30 ± 0.08	0.32 ± 0.07	0.30 ± 0.09	0.37 ± 0.11

Values are given as means ± standard deviation ($n = 8$); S = standard diet (0.5% CLA. 3% $\omega 3$ source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 2% oregano extract; T = Standard diet + 1.5 g/kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/kg prebiotic THEPAX; TO = standard diet + 2% oregano extracts + 1.5 g/kg prebiotic THEPAX. Different superscripts in the same row denote significant differences ($p < 0.01$).

Table 4: Fatty acid composition of rabbit loin belonging to different dietary groups.

Fatty acid (% of total fatty acids)	Rabbit loin					
	Diet					
	S	E	O	T	TE	TO
C14:0	2.3 ± 0.5	1.6 ± 0.4	1.5 ± 0.3	1.8 ± 0.1	2.1 ± 0.2	2.2 ± 0.2
C15:0	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
C16:0	25.8 ± 3.1	22.2 ± 1.6	21.8 ± 1.8	24.2 ± 3.1	24.8 ± 1.2	25.1 ± 0.6
C17:0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.2	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
C18:0	10.1 ± 0.4	11.0 ± 1.4	9.7 ± 0.1	10.4 ± 0.1	10.5 ± 1.1	9.4 ± 1.0
C20:0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
$\sum SFA$	39.9 ± 2.0	36.5 ± 2.4	34.7 ± 1.8	38.4 ± 2.1	39.4 ± 0.3	38.4 ± 0.2
C15:1 Δ^{10c}	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
C16:1 Δ^{9c}	0.9 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.3	0.6 ± 0.2	0.6 ± 0.1
C16:1 *isomer*	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0
C17:1 Δ^{10c}	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
C18:1 Δ^{9t}	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.0
C18:1 Δ^{9c} + C18:1 Δ^{11c}	22.2 ± 1.3	22.7 ± 1.1	23.6 ± 2.3	21.0 ± 2.0	21.0 ± 0.8	22.9 ± 0.4
C20:1 Δ^{11c}	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
$\sum MUFA$	24.5 ± 1.1	24.8 ± 1.1	25.4 ± 1.2	23.6 ± 1.4	23.1 ± 0.6	24.9 ± 0.6
C18:2 $\Delta^{9c,12c}$ [$\omega 6$]	22.8 ± 1.9	24.0 ± 2.1	24.5 ± 2.9	23.7 ± 1.4	23.5 ± 0.4	24.0 ± 0.4
C18:3 $\Delta^{9c,12c,15c}$ [$\omega 3$]	7.3 ± 0.5	8.8 ± 0.0	9.0 ± 0.9	7.5 ± 1.3	7.7 ± 0.3	7.4 ± 0.1
C18:2 $\Delta^{9c,11t}$ [CLA]	1.7 ± 0.2	2.0 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.2	1.5 ± 0.2
C18:2 $\Delta^{10t,12c}$ [CLA]	2.1 ± 0.0	2.4 ± 0.1	2.4 ± 0.2	2.0 ± 0.6	2.2 ± 0.1	1.8 ± 0.1
C20:2 $\Delta^{11,14}$ [$\omega 6$]	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0
C20:3 $\Delta^{8c,11c,14c}$ [$\omega 6$]	0.1 ± 0.0	tr	tr	tr	tr	tr
C20:4 $\Delta^{5c,8c,11c,14c}$ [$\omega 6$]	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.2 ± 0.3	1.2 ± 0.2	1.0 ± 0.1
C20:5	tr	tr	tr	tr	tr	tr
C22:4 $\Delta^{7c,10c,13c,16c}$ [$\omega 6$]	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
C22:5 $\Delta^{7c,10c,13c,16c,19c}$ [$\omega 3$]	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
$\sum CLA$	3.8 ± 0.2	4.4 ± 0.4	4.3 ± 0.3	3.8 ± 0.7	4.1 ± 0.3	3.3 ± 0.4
$\sum PUFA$	35.3 ± 1.9	38.3 ± 1.6	39.3 ± 2.7	38.0 ± 1.6	37.3 ± 0.5	36.3 ± 0.9
PUFA/SFA	0.9 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
PUFA ω6/PUFA ω3	3.2 ± 0.0	2.8 ± 0.2	2.8 ± 0.0	3.4 ± 0.8	3.1 ± 0.2	3.3 ± 0.1

Values are given as means ± standard deviation ($n = 8$); trace (tr), lower than 0.1%; S = standard diet (0.5% CLA. 3% $\omega 3$ source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 2% oregano extract; T = standard diet + 1.5 g/kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/kg prebiotic THEPAX; TO = standard diet + 2% oregano extracts + 1.5 g/kg prebiotic THEPAX.

Table 5: Fatty acid composition of rabbit hind leg belonging to different dietary groups.

Fatty acid (% of total fatty acids)	Rabbit hind leg					
	Diet					
	S	E	O	T	TE	TO
C14:0	1.5 ± 0.4	2.2 ± 0.4	1.3 ± 0.3	2.0 ± 0.3	1.9 ± 0.3	2.0 ± 0.1
C15:0	0.5 ± 0.0	0.9 ± 0.2	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
C16:0	23.3 ± 0.1	23.0 ± 1.4	22.7 ± 0.1	25.5 ± 0.7	24.4 ± 0.6	24.6 ± 1.0
C17:0	0.7 ± 0.0	1.0 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.6 ± 0.1
C18:0	9.9 ± 0.4	10.5 ± 0.1	10.9 ± 0.6	11.1 ± 0.0	10.5 ± 0.7	9.4 ± 0.7
C20:0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
C21:0	0.3 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
$\sum SFA$	36.3 ± 1.0	38.1 ± 2.1	36.4 ± 0.1	40.4 ± 0.9	38.8 ± 1.2	37.5 ± 1.6
C16:1 Δ^{9c}	0.5 ± 0.2	0.8 ± 0.1	0.5 ± 0.0	0.9 ± 0.2	0.6 ± 0.1	0.7 ± 0.1
C16:1 *isomer*	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
C17:1 Δ^{10c}	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
C18:1 Δ^{9t}	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.1
C18:1 Δ^{9c} + C18:1 Δ^{11c}	22.1 ± 0.3	22.5 ± 0.7	19.6 ± 0.9	20.1 ± 0.5	21.1 ± 0.2	20.8 ± 0.8
C20:1 Δ^{11c}	0.3 ± 0.2	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
$\sum MUFA$	23.7 ± 0.3	24.3 ± 1.1	21.0 ± 1.2	21.9 ± 0.5	23.2 ± 0.3	22.5 ± 1.0
C18:2 $\Delta^{9c,12c}$ [ω6]	26.1 ± 1.4	22.8 ± 1.8	25.7 ± 0.7	24.6 ± 1.4	22.9 ± 0.7	24.3 ± 0.4
C18:3 $\Delta^{9c,12c,15c}$ [ω3]	7.0 ± 0.7	6.0 ± 0.7	6.6 ± 0.7	7.0 ± 0.1	6.5 ± 0.8	5.7 ± 0.2
C18:2 $\Delta^{9c,11t}$ [CLA]	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.6	1.6 ± 0.1	1.7 ± 0.3	1.1 ± 0.2
C18:2 $\Delta^{10t,12c}$ [CLA]	1.9 ± 0.2	1.8 ± 0.3	1.7 ± 0.4	1.8 ± 0.4	1.8 ± 0.5	1.3 ± 0.0
C20:2 $\Delta^{11,14}$ [ω6]	0.3 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
C20:3 $\Delta^{8c,11c,14c}$ [ω6]	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
C20:4 $\Delta^{5c,8c,11c,14c}$ [ω6]	1.8 ± 0.5	2.0 ± 0.1	2.5 ± 0.7	1.9 ± 0.3	1.7 ± 0.1	2.3 ± 0.4
C20:5 $\Delta^{5c,8c,11c,14c,17c}$ [ω3]	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
C22:4 $\Delta^{7c,10c,13c,16c}$ [ω6]	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.3 ± 0.0	0.4 ± 0.0	0.7 ± 0.1
C22:5 $\Delta^{7c,10c,13c,16c,19c}$ [ω3]	0.5 ± 0.2	0.7 ± 0.2	1.1 ± 0.4	0.3 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
$\sum CLA$	3.4 ± 0.3	3.4 ± 0.4	3.1 ± 1.0	3.4 ± 0.5	3.5 ± 0.8	2.5 ± 0.2
$\sum PUFA$	39.8 ± 1.1	36.0 ± 3.3	40.7 ± 0.3	37.7 ± 1.8	36.2 ± 2.1	36.8 ± 1.0
PUFA/SFA	1.1 ± 0.1	0.9 ± 0.1	1.1 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.0
PUFA ω6/PUFA ω3	3.8 ± 0.5	3.9 ± 0.3	3.7 ± 0.4	3.6 ± 0.1	3.6 ± 0.3	4.4 ± 0.2

Values are given as means ± standard deviation ($n = 8$); S = standard diet (0.5% CLA. 3% ω3 source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 2% oregano extract; T = standard diet + 1.5 g/kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/kg prebiotic THEPAX; TO = standard diet + 2% oregano extracts + 1.5 g/kg prebiotic THEPAX.

compounds, oregano water extract and vitamin E did not affect the fatty acid relative percentage composition of rabbit meat (% of each fatty acid/total fatty acid). In fact, no statistically significant differences were stated among the fatty acid percentage composition in the samples belonging to different feed groups, both for loin and hind leg. Conversely Dal Bosco et al. [27] found significant changes in fatty acids profile (mainly PUFA) of *longissimus lumborum* meat when rabbits fed diets supplemented with Spirulina (*Arthrospira platensis*) and/or Thyme (*Thymus vulgaris*). Such differences became more significant after 9 days of storage at 4°C, in correspondence to an increase of oxidation.

In all loin and hind leg samples, the level of SFA was comparable to that of PUFA, which accounted for about 35–40% of the total fatty acids. The MUFA portion represented about one-quarter of total fatty acids. The most ubiquitous fatty acids are oleic, palmitic, and linoleic, showing percentages higher than 20%. These findings were in agreement with those of previous works [39, 43, 44].

Although the present results evidenced that addition of oregano extract, prebiotic compounds, and vitamin E into the diet enriched with CLA and PUFA ω3 did not promote the enhancement of bioactive fatty acid content on rabbit meat, it is noteworthy to underline that the adopted dietary strategies lead to rabbit meat (loin and hind leg) with an interesting value of the ratio PUFA ω6/PUFA ω3. It was lower than 4 for each sample and it was markedly lower than those reported in previous works, which consisted of 7 or 11 for loin and hind leg, respectively [26, 44]. As such, the improvement of the PUFA ω6/ω3 ratio to 5 can be considered an important goal

Table 6: PUFA $\omega 3$ content (mg/100 g meat) in rabbit loin and hind leg muscle.

Fatty acid	Diet					
	S	E	O	T	TE	TO
Loin						
C18:3 $\Delta^{9c,12c,15c}$	478 ± 70	411 ± 67	454 ± 55	452 ± 102	348 ± 48	325 ± 74
C22:5 $\Delta^{7c,10c,13c,16c,19c}$	13 ± 7	18 ± 5	11 ± 3	15 ± 2	18 ± 3	9 ± 4
∑PUFA $\omega 3$	491 ± 75[b]	428 ± 68[b]	464 ± 59[b]	457 ± 103[b]	366 ± 50[b]	334 ± 77[b]
Hind leg						
C18:3 $\Delta^{9c,12c,15c}$	221 ± 47	151 ± 31	163 ± 20	165 ± 38	152 ± 67	149 ± 15
C20:5 $\Delta^{5c,8c,11c,14c,17c}$ [EPA]	14 ± 4	15 ± 2	13 ± 1	13 ± 3	10 ± 2	8 ± 6
C22:5 $\Delta^{7c,10c,13c,16c,19c}$ [DPA]	3 ± 1	2 ± 1	4 ± 1	2 ± 1	3 ± 1	3 ± 2
∑PUFA $\omega 3$	238 ± 51[a]	168 ± 34[a]	181 ± 20[a]	180 ± 39[a]	163 ± 68[a]	190 ± 23[a]

Value are given as means ± standard deviation ($n = 8$); S = standard diet (0.5% CLA. 3% n-3 source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 0.2% oregano extract; T = standard diet + 1.5 g/Kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/Kg prebiotic THEPAX; TO = standard diet + 0.2% oregano extracts + 1.5 g/Kg prebiotic THEPAX. Fatty acids of loin are compared with their counterparts of hind leg. Values in the same column with different superscript letters are compared resulting significantly different ($p < 0.01$).

Table 7: Conjugated linoleic acid isomers content (mg/100 g meat) in rabbit loin and hind leg meat samples.

Fatty acids	Diet					
	S	E	O	T	TE	TO
Loin						
C18:2 $\Delta^{9c,11t}$	79 ± 25	110 ± 35	72 ± 19	63 ± 18	59 ± 27	47 ± 12
C18:2 $\Delta^{10t,12c}$	99 ± 19	130 ± 28	101 ± 35	80 ± 14	75 ± 21	60 ± 14
∑CLA	178 ± 45	240 ± 64[b]	173 ± 54[b]	143 ± 32[b]	134 ± 48	107 ± 26[b]
Hind leg						
C18:2 $\Delta^{9c,11t}$	45 ± 14	36 ± 5.7	25 ± 4	26 ± 10	35 ± 5	19 ± 1
C18:2 $\Delta^{10t,12c}$	53 ± 22	42 ± 4	30 ± 7	31 ± 10	37 ± 9	23 ± 4
∑CLA	98 ± 36	78 ± 10[a]	55 ± 11[a]	57 ± 20[a]	71 ± 14	42 ± 4[a]

Values are given as means ± standard deviation ($n = 8$); S = standard diet (0.5% CLA. 3% $\omega 3$ source and 0.5% Vitcon); E = standard diet + 150 ppm vit E; O = standard diet + 2% oregano extract; T = standard diet + 1.5 g/kg prebiotic THEPAX; TE = standard diet + 150 ppm vit E + 1.5 g/kg prebiotic THEPAX; TO = standard diet + 2% oregano extracts + 1.5 g/kg prebiotic THEPAX. Fatty acids of loin are compared with their counterparts of hind leg. Values in the same column with different superscript letters are compared resulting significantly different ($p < 0.01$).

in order to improve the nutritional value of rabbit meat for human benefits [45].

Furthermore, taking into account the strong interest toward the role of rabbit meat as functional food it is noteworthy to examine the absolute amounts (mg/100 g of meat) of bioactive fatty acids, such as PUFA $\omega 3$ and CLA, in loin and hind leg samples obtained by our feeding strategies.

In detail, the PUFA $\omega 3$ absolute amount changed according to meat tissue (Table 6). The loin samples from all dietary groups resulted significantly richer ($p < 0.01$) in PUFA $\omega 3$ than respective hind leg samples. As a result, it was reasonable to suspect that the PUFA $\omega 3$ were preferentially accumulated in loin rather than in hind leg rabbit. However, our results were not consistent with those reported by Petracci et al. [46] who observed as the linseed rich diets provided leg rabbit meat higher in PUFA $\omega 3$ levels than loin rabbit meat: 3% dietary linseed (linseed supplementation equal to that of our standard diet) determined a PUFA $\omega 3$ content of 61 mg/100 g of loin muscle and of 296 mg/100 g of leg meat.

Considering the influence of dietary strategy, the PUFA $\omega 3$ absolute amount weakly changed according to the different feeding. The total PUFA $\omega 3$ amounts revealed in loin meat varied from 334 ± 77 mg/100 g in TO samples to 491 ± 75 mg/100 g in S ones. Concurrently, the total PUFA $\omega 3$ amounts revealed in hind leg meat varied from 163 ± 68 mg/100 g in TE samples to 238 ± 51 mg/100 g in S ones.

Thus, the vitamin E supplementation did not promote the accumulation of PUFA $\omega 3$ in rabbit meat.

The CLA absolute amount changed according to meat tissue (Table 7). The loin samples from E, O, T, and TO groups resulted significantly richer ($p < 0.01$) in CLA than respective hind leg samples. As a result, it was reasonable to suppose that the CLAs were preferentially accumulated in loin rather than in hind leg rabbit. In particular, the highest total CLA content was revealed in loin samples from E supplemented group (240 ± 64 mg/100 g of meat) and in hind leg samples from S group (98 ± 36 mg/100 g of meat).

The CLA fraction of all samples was composed by the same levels of C18:2 $\Delta^{9c,11t}$ and C18:2 $\Delta^{10t,12c}$ CLA isomers. The ratio of these CLA isomers in all samples did not differ from that in the diet. Furthermore, the C18:2 $\Delta^{9t,11c}$ and C18:2 $\Delta^{10c,12t}$ CLA isomers that accounted between 1.9 and 2.6% of total fatty acids of all supplement diet were not detected in loin and hind leg samples.

Differently, Lo Fiego et al. [47] showed that the C18:2 $\Delta^{9c,11t}$ was the predominant isomer in *Longissimus dorsi* muscle of rabbits feed with 0.25 or 0.50% of a CLA preparation containing 65% CLA isomers, half *cis*-9, *trans*-11 and half *trans*-10, and *cis*-12.

4. Conclusions

The experimented dietary strategies, consisting in CLA and PUFA ω3 enriched diets, added with oregano aqueous extract (2 g/kg fed diet) or vitamin E (150 mg/kg feed) or prebiotic compounds from inactivated *S. cerevisiae* yeast were able to obtain functional rabbit meat rich with bioactive fatty acids, such as CLA and PUFA ω3. However, despite the well-known and testified antioxidant activity of oregano and vitamin E, in the present experimental condition, the dietary supplementation with oregano water extracts and/or vitamin E was not able to provide a greater stability of CLA and PUFA ω3 and consequently a selective increase of these fatty acids was not revealed in meat samples.

Similarly, the dietary supplementation with prebiotic compounds did not promote the enhancement of bioactive fatty acid content on rabbit meat. In conclusion, the set of results showed that although variations are observed on the extent of oxidative processes, the fatty acid composition in rabbit meat did not significantly change according to the different dietary strategies, including antioxidant and prebiotic compounds.

Conflicts of Interest

The authors disclose no conflicts of interest.

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